DIETARY EXPOSURE OF MINK (*MUSTELA VISON*) TO FISH FROM THE UPPER HUDSON RIVER, NEW YORK, USA: EFFECTS ON ORGAN MASS AND PATHOLOGY

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Abstract—The authors evaluated effects of feeding ranch mink (*Mustela vison*) diets containing polychlorinated biphenyl (PCB)–contaminated fish from the upper Hudson River (New York, USA) on adult and offspring organ mass and pathology. Diets contained 2.5 to 20% Hudson River fish, providing 0.72 to 6.1 µg $\sum$PCBs/g feed (4.8–38 pg toxic equivalents [TEQWHO 2005]/g feed). Absolute thyroid and adrenal gland masses were increased in adult females and 31-week-old juveniles, respectively, and absolute liver and heart masses were decreased in six-week-old kits exposed to dietary PCBs. Dietary concentrations of 0.72 µg $\sum$PCBs/g feed (4.8 pg TEQWHO 2005/g feed) and greater induced mandibular and maxillary squamous epithelial proliferation in adult animals. The dietary concentration of $\sum$PCBs predicted to result in 20% incidence of jaw lesion (EC20) was 2.3 µg $\sum$PCBs/g feed (15 pg TEQWHO 2005/g feed), and the hepatic concentration was 2.8 µg $\sum$PCBs/g liver (89 pg TEQWHO 2005/g liver, wet weight). The EC20 values were greater than the dietary and hepatic concentrations predicted to result in a 20% increase in kit mortality (LC20) at six weeks of age (0.34 µg $\sum$PCBs/g feed or 2.6 pg TEQWHO 2005/g feed and 0.80 µg $\sum$PCBs/g liver or 13 pg TEQWHO 2005/g liver). However, the EC20 values reflect exposure of adults to PCBs for approximately six months, and the lethal concentration 20% values reflect exposure of offspring from conception onward.

Keywords—Hudson River, Mink, Polychlorinated biphenyl, Organ weight, Pathology
INTRODUCTION

The Hudson River flows 507 km from its source in the Adirondack Mountains to its confluence with New York Harbor at the Battery in Manhattan, New York, USA. The river is contaminated with polychlorinated biphenyls (PCBs) from Fort Edward, New York, to New York City, a distance of approximately 285 km. The source of the PCBs is attributed to two plants in Fort Edward and Hudson Falls, New York, that manufactured electrical capacitors containing PCBs. It is estimated that in the course of manufacturing activities, almost 600 metric tons of PCBs were discharged into the Hudson River between the 1940s and 1977, accounting for 50% of the PCB contamination in New York Harbor [1,2].

A number of mammalian species, including the mink (Mustela vison), depend on the Hudson River and its floodplain for habitat, food, and breeding sites. As a piscivorous mammal, the mink is at risk from exposure to PCBs and related contaminants that have entered the aquatic environment and bioconcentrated within the food chain. Reports spanning the past 20 years have indicated that mink collected within one home range (6 km) of the upper Hudson River contained concentrations of PCBs in their fat and livers comparable to concentrations causing reproductive impairment in controlled studies with ranch mink and that tissue concentrations of PCBs have not decreased appreciably [3,4].

Because of the mink’s sensitivity to PCBs [5–7] and its designation as a sentinel wildlife species [8], a study was designed to evaluate the health effects of feeding farm-raised mink diets containing PCB-contaminated fish collected from the Hudson River. One objective, which is addressed in a companion article [9, this issue] was to assess effects on adult reproductive performance (percentage of females whelping, gestation length, percentage of stillbirths, and live kits whelped) and offspring growth and mortality through 31 weeks of age. A second objective, which is addressed in the present article, was to determine if exposure to PCB-contaminated fish would affect mass and
morphology of specific organs and tissues in adult mink and their offspring. Of particular interest was a jaw lesion characterized as mandibular and maxillary squamous epithelial proliferation. This lesion has been described in a number of controlled studies involving exposure of mink to specific 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)–like congeners including 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126 using International Union of Pure and Applied Chemistry nomenclature) [10–12] and environmentally derived mixtures of PCBs, polychlorinated dibenzo-p-dioxin (PCDDs), and polychlorinated dibenzofurans (PCDFs) through incorporation of contaminated fish into the diet [13,14]. In addition, the lesion has been reported in wild mink collected from sites contaminated with PCB, PCDD, and PCDF [15,16]. As the jaw lesion appears to be associated with exposure to TCDD-like chemicals, can be induced in a laboratory setting in a sentinel species, and is known to occur in the wild, there is interest in mandibular and maxillary squamous epithelial proliferation as a potential indicator of exposure and adverse effects.

This article presents the effects of feeding diets containing PCB-contaminated fish from the Hudson River on the mass and morphology of specific organs and tissues, including the mandible and maxilla, of adult farm-raised mink and their offspring in terms of dietary and hepatic concentrations of $\Sigma$PCBs and World Health Organization (WHO) TCDD toxic equivalents (TEQ$_{\text{WHO,2005}}$).

**METHODS**

The mink feeding study, approved by the Michigan State University Institutional Animal Care and Use Committee, has been previously described [9, this issue]. Briefly, 1,521 kg of carp (Cyprinus carpio) collected from three different locations in the upper Hudson River were ground and incorporated into a basal mink diet [9] at 0, 2.5, 5.0, 10, 15, and 20% of the total diet. The upper limit of 20% dietary Hudson River fish was based on the mean $\Sigma$PCB concentration in the collected fish (36 $\mu$g $\Sigma$PCBs/g) and the desire to not cause complete reproductive failure and/or adult mortality. Measured mean $\Sigma$PCB concentrations (±standard deviation) were 0.0074 (0.0016), 0.72 (0.12), 1.5
(0.21), 2.8 (0.38), 4.5 (0.49), and 6.1 (0.51) µg/g feed, respectively. The TEQ\textsubscript{WHO 2005} concentrations (±standard deviation), based on toxic equivalency factors (TEFs\textsubscript{WHO 2005}) [17], were 0.41 (0.022), 4.8 (0.15), 10 (0.074), 18 (0.13), 28 (0.12), and 38 (0.38) pg/g feed, respectively, as presented in Bursian et al. [9, this issue]. All concentrations are expressed on a wet weight basis unless otherwise specified.

A total of 75 first-year (virgin), natural dark, female mink and 30 first-year, natural dark, male mink from the Michigan State University Experimental Fur Farm herd were randomly assigned to the six treatment groups. The control and 4.5- and 6.1-µg \(\sum\)PCBs/g feed groups had 15 females and five males each. The 0.72-, 1.5-, and 2.8-µg \(\sum\)PCBs/g feed groups had 10 females and five males each. Mink were fed the experimental diets for approximately 170 days, beginning two months before breeding and continuing through gestation, lactation, and early kit growth. Those kits not necropsied were maintained on their respective treatment diets for an additional 170 days. Body weights were determined on a monthly basis until termination of the trial, when the juvenile mink were approximately 31 weeks old.

Adults and a subsample of kits were necropsied when kits were six weeks old, and surviving juveniles were necropsied at 31 weeks of age. The number of surviving kits and juveniles at key time points during the study and the number of kits and juveniles necropsied are presented in Table 1.

At time of necropsy, animals were weighed and euthanized with CO\textsubscript{2}, and specific tissues were collected for subsequent analyses. The liver, brain, heart, kidneys, spleen, thyroid gland, adrenal glands, and testes or uterus (adults only) were removed, weighed, and placed in buffered formalin for histological examination. A portion of the liver also was collected for determination of \(\sum\)PCB and TEQ\textsubscript{WHO 2005} concentrations, as described in Bursian et al. [9, this issue]. After removal of the brain, the mandible and maxilla were detached from the rest of the skull, placed in buffered formalin, and processed and evaluated as described in Beckett et al. [15]. The primary pathologist as well as an independent pathologist conducted a blind review of the slides.
Statistical analyses of measurement end points were conducted using a generalized linear model framework [18], where the most appropriate class of linear models was selected based on classification of data type and correlation structure. A summary of end points classified by data type and analysis method is provided in Table 2. Continuous end points were analyzed by a linear regression model [19] when the end point was measured at the experimental unit level and the experimental units within a treatment group were expected to be independent (e.g., adult mink). Continuous end points having kits clustered within litters were analyzed by linear generalized estimating equation (GEE) regression. The GEE models are a contemporary extension of generalized linear models for clustered data that adjust for within-cluster correlation [20]. Examples of clustered data in the present study were kit organ masses within litters. Binary end points measured on adults (e.g., presence of jaw lesions) were analyzed with logistic regression or, in the case of complete separation (i.e., for at least one dietary concentration end point values were the same), with Fisher’s exact test [21]. For histological end points having significant treatment effects, the effective concentration resulting in 20 (EC20) and 50% (EC50) prevalence of that pathology was estimated with logistic regression. Dose–response relationships were estimated for dietary and hepatic ΣPCBs as well as TEQ<sub>WHO 2005</sub>. Binary end points measured on kits were analyzed with beta-binomial models or, in the case of complete separation, with Fisher’s exact test. Beta-binomial models were used to account for the variation that occurred both between kits in the same litter (binomial variation) and between litters in the same treatment group (extrabinomial variation).

All statistical analyses were conducted using R statistical software (http://www.r-project.org/), including the additional R packages geepack for GEE models and doBy for linear functions of estimated GEE regression parameters.
RESULTS

Organ masses

Absolute brain, kidney, spleen, heart, and adrenal gland masses of treated adult female mink were not significantly different from the control group at necropsy. Means for absolute thyroid gland mass of adult females in the 4.5- and 6.1-µg $\sum$PCBs/g feed groups were 0.0083 g (17%) greater (confidence interval [CI] 0.00058–0.016 g, $p = 0.036$) and 0.0078 g (16%) greater (CI 0.00010–0.016 g, $p = 0.047$), respectively, than the female control mean (0.049 g, CI 0.043–0.054 g). Results for organ masses for adult males were not reported because of the small number of males in the study.

For six-week-old kits, heart and liver masses were decreased relative to controls, but differences were not significant for other organs. Organ masses of six-week-old kits were measured for a total of 37 animals (seven females and eight males in the control group, five females and four males in the 0.72-µg $\sum$PCBs/g feed group, and six females and seven males in the 1.5-µg $\sum$PCBs/g feed group). No kits from the 2.8-, 4.5-, and 6.1-µg $\sum$PCBs/g feed groups were necropsied. Means for absolute heart mass of six-week-old kits in the 0.72- and 1.5-µg $\sum$PCBs/g feed groups were 0.35 g (19%) less (CI –0.67 to –0.032 g, $p = 0.031$) and 0.39 g (22%) less (CI –0.67 to –0.12 g, $p = 0.0050$), respectively, than male and female means for control heart mass (male = 1.90 g, CI 1.7–2.1 g; female = 1.7 g, CI 1.5–1.8 g); and the treatment effects were not significantly modified by gender. Mean absolute liver mass of six-week-old kits in the 1.5-µg $\sum$PCBs/g feed group was 3.8 g (19%) less (CI –7.3 to –0.30 g, $p = 0.033$) than the male and female means for control liver mass (male = 21 g, CI 19–23 g; female = 19 g, CI 16–21 g), and the treatment effect was not significantly modified by gender.

For the few mink surviving to 31 weeks of age, the only organ mass that was different between the remaining treatment group and the control group was mass of the adrenal gland. Mean absolute adrenal gland mass in the 0.72-µg $\sum$PCBs/g feed group was 0.014 g (17%) greater (CI 0.0017–0.26 g, $p = 0.026$) than the male and female means for control adrenal glands mass (male = 0.10 g, CI 0.10–0.11 g; female = 0.08 g, CI 0.07–0.09 g).
g; female = 0.068 g, CI 0.060–0.070 g), and the treatment effect was not significantly modified by gender.

**Organ histopathology**

Mandibles and maxillae of adult mink had lesions that were more frequent and severe in the groups exposed to the greatest concentrations of $\sum$PCBs. Mandibles and maxillae were normal for 65 of 100 adults, including all 20 controls. The lesion that occurred in the mandible and maxilla was characterized by the presence of squamous cell hyperplastic foci (Fig. 1). Mild lesions (Fig. 1B) were present in one animal in the 0.72-µg $\sum$PCBs/g feed group, two animals each in the 1.5- and 2.8-µg $\sum$PCBs/g feed groups, five animals in the 4.5-µg $\sum$PCBs/g feed group, and seven animals in the 6.1-µg $\sum$PCBs/g feed group. Mandibular and maxillary lesions of moderate severity (Fig. 1C) occurred in two 1.5-µg $\sum$PCBs/g feed animals, five 2.8-µg $\sum$PCBs/g feed animals, and seven 6.1-µg $\sum$PCBs/g feed animals. Severe lesions (Fig. 1D) occurred in three 6.1-µg $\sum$PCBs/g feed adults. The frequency of lesions in the mandibles and maxillae differed significantly among treatment groups (Fisher’s exact test $p < 0.001$). The percentage of adults with nonnormal jaw tissue increased monotonically from that of the control group to that for the 6.1-µg $\sum$PCBs/g feed group: 0% (0/20, control), 7.1% (1/14, 0.72-µg $\sum$PCBs/g feed group), 13.3% (2/15, 1.5-µg $\sum$PCBs/g feed group), 28.6% (4/14, 2.8-µg $\sum$PCBs/g feed group), 66.7% (12/18, 4.5-µg $\sum$PCBs/g feed group), 84.2% (16/19, 6.1-µg $\sum$PCBs/g feed group) (Fig. 2).

In adult mink, no lesions were observed in brains, hearts, thyroid glands, ovaries, uteri, or testes; but vacuolation and mineralization were present in livers, kidneys, and adrenals, respectively, of mink from both control and treatment groups. Moderate hepatic vacuolation was observed in 91 of 99 liver samples assessed including 19 of 20 control samples. Mild vacuolation was observed in one liver each in the control, 2.8-, and 4.5-µg $\sum$PCBs/g feed groups and two livers in the 0.72-µg $\sum$PCBs/g feed group. Severe vacuolation was observed in two livers in the 1.5-µg $\sum$PCBs/g feed group and one in the
4.5-µg $\sum$PCBs/g feed group. The frequency of severe vacuolation did not differ significantly between treatment groups (Fisher’s exact test $p = 0.15$). Mild mineralization was observed in 98 of 100 kidney samples including all 20 controls. One sample in the 6.1-µg $\sum$PCBs/g feed group was normal, and one kidney sample in the 0.72-µg $\sum$PCBs/g feed group had moderate mineralization. The frequency of mineralization in renal tissue did not differ significantly among treatment groups (Fisher’s exact test $p = 0.28$). Adrenal glands were normal for 87 of 99 adults. Mild mineralization was observed in one sample each in the control and 1.5-µg $\sum$PCBs/g feed groups, two samples each in the 2.8- and 4.5-µg $\sum$PCBs/g feed groups, and three samples each in the 0.72- and 6.1-µg $\sum$PCBs/g feed groups. The frequency of mineralization in adrenal tissue did not differ significantly among treatment groups (Fisher’s exact test $p = 0.71$).

Lesions were observed in the spleens of adult mink, but these lesions did not appear to be treatment-related. Spleens were normal for 96 of 99 adults, including the 20 control animals. Focal hemosiderosis was reported for two spleen samples in the 0.72-µg $\sum$PCBs/g feed group and one sample in the 4.5-µg $\sum$PCBs/g feed group. The frequency of lesions in spleen tissue did not differ significantly among treatment groups (Fisher’s exact test $p = 0.12$).

In six-week-old kits, the pathologies observed were not severe and did not differ significantly in frequency among the control kits and those sampled in the 0.72- and 1.5-µg $\sum$PCBs/g feed groups. All brain, heart, spleen, adrenal gland, and thyroid gland samples were normal; and all liver samples had moderate vacuolation. Twenty-four of 37 kidney samples had mild mineralization (8 of 15 in the control group, 6 of 9 in the 0.72-µg $\sum$PCBs/g feed group, and 10 of 13 in the 1.5-µg $\sum$PCBs/g feed group). The frequency of renal mineralization did not differ significantly among treatment groups (Fisher’s exact test $p = 0.42$). Mandibles and maxillae were normal for 34 of 37 kits, including 14 of 15 in the control group. Mild squamous cell hyperplasia was reported for one kit each in the control and 1.5-µg $\sum$PCBs/g feed groups and moderate squamous cell hyperplasia in one kit in the 0.72-µg
∑PCBs/g feed group. The frequency of jaw pathology did not differ significantly among treatment groups (Fisher’s exact test \( p \approx 1 \)).

Similar to those of six-week-old kits, the tissues of the juveniles surviving at 31 weeks of age did not have treatment-related pathologies. All brain, heart, thyroid gland, and mandible/maxilla samples were normal; and all renal samples had mild mineralization. Moderate hepatic vacuolation was apparent in samples from 28 of 30 juvenile controls and 22 of 23 juveniles in the 1.5-µg ∑PCBs/g feed group. The remaining three liver samples had mild vacuolation. The frequency of vacuolation did not differ between the two treatment groups (Fisher’s exact test \( p \approx 1 \)). One spleen from the 0.72-µg ∑PCBs/g feed group had mild multifocal hemosiderosis, with the frequency being nonsignificant (Fisher’s exact test \( p = 0.43 \)). Mild mineralization of the adrenal gland occurred in two control samples and one sample from the 0.72-µg ∑PCBs/g feed group, with the remaining 50 samples being normal. The frequency of pathology in adrenal tissue did not differ significantly between treatment groups (Fisher’s exact test \( p \approx 1 \)).

**Effective concentrations**

Estimated dietary and hepatic EC20 and EC50 values for the incidence of jaw lesions in adult mink were derived in terms of ∑PCB and TEQWHO 2005 concentrations (Table 3). Plots illustrating derivation of EC20 and EC50 values based on dietary ∑PCB and TEQWHO 2005 concentrations and hepatic ∑PCB and TEQWHO 2005 concentrations are presented in Figures 3 and 4, respectively.

**DISCUSSION**

**Organ masses**

The thyroid gland is a target organ of TCDD-like chemicals, including PCB 126 [22], which contributed 74% of the TEQWHO 2005 in the present study [9, this issue]. Similar to the increase in absolute thyroid mass in adult females at the two greatest dietary concentrations reported in the present study, adult female mink fed diets containing PCB-contaminated fish from Saginaw Bay (Lake Huron,
Michigan, USA) had a significant increase in the relative mass (expressed as percentage of brain mass) of the thyroid gland at 1.5-µg $\Sigma$PCBs/g feed and greater [23]. The TCDD-like chemicals induce hepatic UDP-glucuronosyltransferase 1, resulting in increased glucuronidation of thyroxine and subsequent biliary excretion of thyroxine-glucuronide. The decrease in circulating thyroxine results in increased secretion of thyroid-stimulating hormone, which in turn leads to thyroid follicular cell hyperplasia and hypertrophy [22]. In the present study, however, histological examination of the thyroid gland did not indicate hyperplasia or hypertrophy, and thyroid hormone concentrations were not determined.

Exposure to TCDD-like chemicals results in changes in heart size during embryonic/fetal development in fish, birds, and mammals [24]. The decrease in absolute heart mass of six-week-old kits reported in the present study is similar to results of the Saginaw Bay study [23] in that in the latter study, there was a general decrease in the relative masses of all organs examined in kits surviving until weaning (0.72- and 1.5-µg $\Sigma$PCBs/g feed groups only). The reduction in fetal heart size in the mouse is manifested as cardiac remodeling and hypertrophy, decreased heart rate, and a predisposition to cardiovascular disease in adulthood [24]. Despite the increase in absolute heart mass, there was no evidence of morphological changes in cardiac tissue in the present study.

The decrease in absolute liver mass in six-week-old kits reported in the present study is opposed to hepatomegaly, which is the typical response in a variety of mammalian species exposed to TCDD-like chemicals. Predominant histological features of hepatomegaly, which are accompanied by altered liver function, include hepatocellular hypertrophy, multinuclear hepatocytes, steatosis, and inflammatory cell infiltration [25]. Increases in liver mass in mink exposed to PCBs were reported in the Saginaw Bay study [23], where the relative mass (expressed as percentage of brain mass) of adult liver was significantly increased at dietary concentrations of 0.72-µg $\Sigma$PCBs/g feed and greater. Similarly, Restum et al. [26] reported an increase in absolute and relative (percentage of brain mass).
liver mass of male mink fed a diet containing 1.0 µg $\sum$PCBs/g feed derived from fish collected from Saginaw Bay and an increase in absolute liver mass in males fed 0.5 µg $\sum$PCBs/g feed for 18 months. Six-week-old kits whelped by females fed a diet containing fish collected from the Housatonic River in Massachusetts, USA, that provided 3.7 µg $\sum$PCBs/g feed had increased relative liver mass [14]. The decrease in absolute liver masses of six-week-old kits reported in the present study is similar to results reported by Heaton et al. [23] in that in the latter study, there was a general decrease in the relative masses of all organs examined in kits surviving until weaning (0.72- and 1.5-µg $\sum$PCBs/g feed groups only). The somewhat unexpected decrease in liver mass reported in the present study, as well as in the Saginaw Bay study [23], could be a reflection of very small kits. Six-week-old kits in the 1.50-$\sum$PCBs/g feed group weighed 21% less compared to controls, and none survived to 31 weeks of age [9]. The lack of treatment-related hepatic pathology suggests that the change in absolute liver mass reported in the present study is not physiologically important.

Increased mass of the adrenal gland has been reported as a result of exposure to TCDD in a number of mammalian species [27]. The only other report of increased adrenal gland mass reported in mink studies, in addition to the increased absolute mass in 31-week-old juveniles exposed to 0.72 µg $\sum$PCBs/g feed reported in the present study, was an increase in relative mass of the adrenal glands collected from adult females exposed to 1.5 µg $\sum$PCBs/g feed and greater in the Saginaw River study [23]. In guinea pigs exposed to TCDD, an increase in adrenal gland mass was associated with areas of hemorrhage in the zona reticularis and medulla, cellular necrosis throughout the gland, and atrophy of the zona glomerulosa [27]. In the present study, the reported increase in adrenal gland mass was not associated with treatment-related pathologies.

*Organ histopathology and etiology*

Similar to other mink feeding studies [13,14], the only significant treatment-related pathology in the present study was related to the mandible and maxilla. Proliferation of squamous epithelial cells in
the mandible and maxilla of adult mink was observed at the lowest dietary concentration (0.72 µg ∑PCBs/g feed) and increased in frequency and severity with increasing dietary concentrations.

This particular lesion in mink was initially reported by Render et al. [10], who observed maxillary and mandibular osteoinvasive squamous epithelial proliferation in 12-week-old mink fed a diet containing PCB 126. In the present study, PCB 126 contributed 74% of the TEQ_{WHO 2005} [9]. Subsequent studies by Render et al. [11,12] verified induction of the lesion by PCB 126 and demonstrated its induction by TCDD in both young mink as well as adults. Juvenile mink exposed from conception onward to environmentally derived PCBs through incorporation of fish into the diet developed the lesion as reported for the Saginaw River [13] and Housatonic River [14] studies. The no observed adverse effect levels (diet-based)/concentrations (tissue-based) (NOAELs/NOAECs) and lowest observed adverse effect levels/concentrations (LOAELs/LOAECs) for these studies as well as the present study are presented in Table 4. The difference in NOAELs/NOAECs ranged from 15-fold (hepatic TEQ_{WHO 2005}) to 153-fold (hepatic ∑PCBs), and differences in LOAELs/LOAECs ranged from 1.8-fold (dietary ∑PCBs) to 22-fold (hepatic ∑PCBs). In a recent field study, Beckett et al. [15] reported histological evidence of a jaw lesion characterized by hyperplasia of squamous epithelium in the mandible and maxilla in wild mink collected during an assessment of PCB exposure in the Kalamazoo River basin located in southwestern Michigan [28,29]. Lesion severity was positively correlated with hepatic ∑PCB (2.9–6.0 µg/g liver) and TEQ_{WHO 2005} (210–1,300 pg/g liver) concentrations in mink collected from the study area. More recently, Haynes et al. [16] reported that a mink collected near the shore of Lake Ontario within the Rochester Embayment area of concern, New York, had histological and gross evidence of mandibular and maxillary squamous epithelial proliferation. This animal had a hepatic ∑PCB concentration of 5.9 µg/g liver and a TEQ_{WHO 2005} concentration (based on PCDDs and PCDFs) of 21 pg/g liver.
Effective concentrations

Because there are acknowledged limitations associated with use of NOAELs/NOAECs and LOAELs/LOAECs [30,31] in risk assessment, EC20 and EC50 values were derived for the incidence of jaw lesions in the present study. Regression estimates of lethal concentrations (EC20 and EC50) are less sensitive to the idiosyncrasies of experimental design, and estimates are not limited to the concentrations under study. The only other mink feeding study that reported EC values for the incidence of jaw lesions was the Housatonic River study [14]. It is important to point out that the Housatonic River EC values are not directly comparable in the EC values in the present study because the Hudson River EC values are based on lesions occurring in adult animals exposed to PCBs for approximately 170 days, while the EC values in the Housatonic River study [14] are based on lesions occurring in juvenile mink exposed from conception through 31 weeks of age (adults were not evaluated). It has been suggested that young mink are more susceptible to proliferation of periodontal squamous epithelium than adult mink because of enhanced epithelial cell activity in the jaws during development [12], although a difference in susceptibility based on age has not been systematically evaluated.

It is interesting to note that the jaw lesion in the present study was detected in three six-week-old kits but not in the 31-week-old juveniles. Mild squamous cell hyperplasia was reported for one kit each in the control and 1.5-µg ΣPCBs/g feed groups and moderate squamous cell hyperplasia in one kit in the 0.72-µg ΣPCBs/g feed group. None of the kits in the 1.5-µg ΣPCBs/g feed group survived to 31 weeks of age; thus, the progressive development of the lesion in this group could not be assessed. Since no lesions were detected in surviving juveniles in the 0.72-µg ΣPCBs/g feed group, it is possible that susceptible animals died prior to evaluation at the end of the study.

Despite the differences between the two studies in exposure scenario for animals having the jaw lesion as described above, the EC values in the present study were similar to the Housatonic River EC
values (Table 4). Hudson River dietary EC values were generally less than twice the corresponding
EC values from the Housatonic River study (Hudson River dietary and hepatic EC50 values were
approximately 2.1 and 1.4 times greater, respectively, than estimated Housatonic River values; and
Hudson River dietary and hepatic EC20 values were approximately 1.7 and 1.2 times greater,
respectively, than corresponding Housatonic River values).

In the Hudson River study, the EC20 values for incidence of jaw lesions were approximately
5.5-fold greater than corresponding LC20 values for kit mortality in the present study, as described [9].
It should be noted that in the present study, the LC value reflects effects in the offspring exposed to
PCBs from conception onward, whereas the EC value reflects effects in adults exposed for a discrete
period of time. In the Housatonic River study, the EC20 for the incidence of jaw lesions based on
dietary ∑PCBs (Table 4) was 1.3 times greater than the corresponding LC20 for kit mortality, as
described [9], while the LC20 for kit mortality based on dietary TEQWHO 2005 [9, this issue] was 1.6
times greater than the corresponding EC20 for the incidence of jaw lesions. In the Housatonic River
study [14], the LC and EC values were based on effects in the offspring exposed to PCBs from
conception onward.

Results of the present study indicate that consumption of feed containing fish collected from the
Hudson River induced development of a jaw lesion in adult mink characterized as mandibular and
maxillary squamous epithelial proliferation. A dietary concentration of 2.3 µg ∑PCBs/g feed (15 pg
TEQWHO 2005/g feed) was predicted to result in a 20% incidence of the jaw lesion. Assuming that mink
residing along the upper Hudson River consume fish containing an average of 4.0 µg ∑PCBs/g [9], that
fish is the only component of the mink’s diet that contains PCBs or other aryl hydrocarbon receptor–
active compounds, and that the animal resides in the area for at least six months, a daily diet consisting
of 60% fish would provide a dietary concentration of ∑PCBs that resulted in a 20% incidence of the
jaw lesion in the present study.
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Fig. 1. Mandibular and maxillary squamous epithelial proliferation in adult mink exposed to polychlorinated biphenyls (PCBs) derived from Hudson River fish for approximately 170 days.  

(A) Photomicrograph of a mandible from a control animal (lesion severity score = 0). Notice the tooth (T) embedded in alveolar bone (AB).  

(B) Photomicrograph of a mandible from an animal exposed to 4.5 µg ΣPCBs/g feed (lesion severity score = 1). Arrow indicates an area of squamous epithelial cell proliferation.  

(C) Photomicrograph of a mandible from a mink fed a diet containing 4.5 µg ΣPCBs/g feed (lesion severity score = 2). Arrows indicate multiple cysts of squamous epithelial cells.  

(D) Photomicrograph of a mandible from a mink fed a diet containing 6.1 µg ΣPCBs/g feed (lesion severity score = 3). Arrows indicate cysts of squamous epithelial cells surrounding the tooth.

Fig. 2. Effect of exposure to polychlorinated biphenyls (PCBs) derived from Hudson River fish on the incidence (%) and severity of squamous epithelial proliferation in adult mink. Lesion severity was scored from 0 (normal) to 3 (severe) as described by Beckett et al. [15].

Fig. 3. Effective concentrations, EC20 and EC50, with 95% confidence interval (shaded horizontal interval) based on Σpolychlorinated biphenyls (PCBs) in feed (micrograms per gram of feed, top) and toxic equivalents (TEQ WHO 2005) in feed (picograms per gram of feed, bottom) for presence of jaw lesions in adult mink resulting from consumption of diets containing PCB-contaminated fish collected from the Hudson River. Sample size was 100 adults, consisting of 72 females and 28 males.

Fig. 4. Effective concentrations, EC20 and EC50, with 95% confidence interval (shaded horizontal interval) based on hepatic Σpolychlorinated biphenyls (PCBs) (micrograms per gram liver, wet wt; top) and toxic equivalents (TEQ WHO 2005) (picograms per gram liver, wet wt; bottom) for presence of jaw lesions in adult mink exposed to PCBs derived from Hudson River fish. Sample size was 100 adults, consisting of 72 females and 28 males.
Table 1. Number of surviving kits/juveniles at key time points during the study

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed group based on dietary concentration</td>
<td>Whelped alive</td>
<td>Weaned (approximately 6 weeks old)</td>
<td>Alive and available for necropsy</td>
<td>Necropsied</td>
<td>Alive post necropsy period</td>
<td>Alive at start of growth trial</td>
<td>Alive at end of growth trial</td>
<td>Necropsied at end of growth trial</td>
</tr>
<tr>
<td>Control</td>
<td>83</td>
<td>66</td>
<td>62</td>
<td>15</td>
<td>47</td>
<td>47</td>
<td>47</td>
<td>30</td>
</tr>
<tr>
<td>0.78 µg ΣPCBs/g feed</td>
<td>49</td>
<td>39</td>
<td>33</td>
<td>9</td>
<td>24</td>
<td>24</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>1.5 µg ΣPCBs/g feed</td>
<td>72</td>
<td>51</td>
<td>40</td>
<td>13</td>
<td>27</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2.8 µg ΣPCBs/g feed</td>
<td>46</td>
<td>9</td>
<td>8</td>
<td>0</td>
<td>8</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4.5 µg ΣPCBs/g feed</td>
<td>40</td>
<td>15</td>
<td>9</td>
<td>0</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6.1 µg ΣPCBs/g feed</td>
<td>31</td>
<td>2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>


PCB = polychlorinated biphenyl.
Table 2. Summary of study end points, data types, and statistical methods

<table>
<thead>
<tr>
<th>End point</th>
<th>Data type</th>
<th>Statistical methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult female organ masses</td>
<td>Continuous</td>
<td>Linear regression/ANOVA</td>
</tr>
<tr>
<td>Six-week-old kit organ masses</td>
<td>Continuous</td>
<td>Linear generalized estimating equation regression</td>
</tr>
<tr>
<td>Seven-month-old juvenile organ masses</td>
<td>Continuous</td>
<td>Linear generalized estimating equation regression</td>
</tr>
<tr>
<td>Histopathology of adult organs and jaws</td>
<td>Binary</td>
<td>Logistic/Fisher's exact test</td>
</tr>
<tr>
<td>Histopathology of six-week-old kit organs and jaws</td>
<td>Binary</td>
<td>Logistic regression/Fisher's exact test</td>
</tr>
<tr>
<td>Histopathology of seven-month-old juvenile organs and jaws</td>
<td>Binary</td>
<td>Beta-binomial regression/Fisher's exact test</td>
</tr>
</tbody>
</table>

ANOVA = analysis of variance.
Table 3. Concentrations of ∑PCBs (micrograms per gram of feed or liver) and toxic equivalents (TEQWHO 2005, picograms per gram of feed or liver) resulting in 20% (EC20) and 50% (EC50) incidence of mandibular and maxillary squamous epithelial proliferation in adult mink exposed for approximately 170 days

<table>
<thead>
<tr>
<th>Response</th>
<th>Covariate</th>
<th>EC20</th>
<th>LCL</th>
<th>UCL</th>
<th>EC50</th>
<th>LCL</th>
<th>UCL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jaw lesions</td>
<td>∑PCBs (µg/g feed)</td>
<td>2.3</td>
<td>1.5</td>
<td>3.1</td>
<td>3.9</td>
<td>3.2</td>
<td>4.6</td>
</tr>
<tr>
<td>Jaw lesions</td>
<td>TEQWHO 2005 (pg/g feed)</td>
<td>15</td>
<td>10</td>
<td>20</td>
<td>24</td>
<td>20</td>
<td>28</td>
</tr>
<tr>
<td>Jaw lesions</td>
<td>∑PCBs (µg/g liver, wet wt)</td>
<td>2.8</td>
<td>2.1</td>
<td>3.6</td>
<td>4.4</td>
<td>3.7</td>
<td>5.1</td>
</tr>
<tr>
<td>Jaw lesions</td>
<td>TEQWHO 2005 (pg/g liver, wet wt)</td>
<td>89</td>
<td>58</td>
<td>121</td>
<td>151</td>
<td>125</td>
<td>178</td>
</tr>
</tbody>
</table>

aLogistic regression was used to derive the EC20 and EC50 values.
bHepatic concentrations of ∑PCBs and TEQWHO 2005 used are those for adult males and females combined. The ∑PCB concentrations are 0.053, 1.2, 2.7, 3.1, 4.9, and 6.6 µg/g liver; and TEQWHO 2005 concentrations are 2.2, 29, 61, 99, 184, and 231 pg/g liver, wet wt, for the control and 0.72-, 1.5-, 2.8-, 4.5-, and 6.1-µg ∑PCBs/g feed groups, respectively.

PCB = polychlorinated biphenyl; CI = confidence interval; LCL = lower confidence limit; UCL = upper confidence limit.
Table 4. Comparison of threshold concentrations of $\sum$PCBs (micrograms per gram of feed or liver) and toxic equivalents (TEQ$_{\text{WHO 2005}}$, picograms per gram of feed or liver) relating to mandibular and maxillary squamous epithelial proliferation

<table>
<thead>
<tr>
<th>Threshold type</th>
<th>Location</th>
<th>Animal age$^a$</th>
<th>$\sum$PCBs (µg/g feed)</th>
<th>TEQ$_{\text{WHO 2005}}$ (µg/g liver)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOAEL/NOAEC</td>
<td>Hudson River</td>
<td>Adult</td>
<td>0.0074</td>
<td>0.41</td>
<td>0.053</td>
</tr>
<tr>
<td></td>
<td>Saginaw River</td>
<td>27-week juveniles</td>
<td>0.83</td>
<td>22</td>
<td>8.1</td>
</tr>
<tr>
<td></td>
<td>Housatonic River</td>
<td>31-week juveniles</td>
<td>0.96</td>
<td>6.6</td>
<td>1.7</td>
</tr>
<tr>
<td>LOAEL/LOAEC</td>
<td>Hudson River</td>
<td>Adult</td>
<td>0.72</td>
<td>4.8</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>Saginaw River</td>
<td>27-week juveniles</td>
<td>1.1</td>
<td>36</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Housatonic River</td>
<td>31-week juveniles</td>
<td>0.61</td>
<td>4.2</td>
<td>0.73</td>
</tr>
<tr>
<td>EC50</td>
<td>Hudson River</td>
<td>Adult</td>
<td>3.9</td>
<td>24</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>Housatonic River</td>
<td>31-week juveniles</td>
<td>1.8</td>
<td>13</td>
<td>3.9</td>
</tr>
<tr>
<td>EC20</td>
<td>Hudson River</td>
<td>Adult</td>
<td>2.3</td>
<td>15</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>Housatonic River</td>
<td>31-week juveniles</td>
<td>1.3</td>
<td>9.7</td>
<td>2.7</td>
</tr>
</tbody>
</table>

$^a$Adults were exposed to $\sum$PCBs for approximately 170 days, and juveniles were exposed from conception until 27 or 31 weeks of age. Adult animals were not evaluated for the incidence of jaw lesion in the Saginaw River and Housatonic River studies.

PCB = polychlorinated biphenyl; NOAEL/NOAEC = no observed adverse effects level/concentration; LOAEL/LOAEC = lowest observed adverse effect level/concentration; EC50 = concentration resulting in 50% incidence of the lesion; EC20 = concentration resulting in 20% incidence of the lesion.
Figure 1.
Figure 2

% Incidence of severity score

Concentration (µg ΣPCBs/g feed)

- Lesion score 3
- Lesion score 2
- Lesion score 1
Figure 3

EC20 for jaw lesion in adult mink

EC50 for jaw lesion in adult mink

\[ P_{\text{Lesion}} = \frac{e^{-3.41-0.87x}}{1 + e^{-3.41-0.87x}} \]

\[ P_{\text{Lesion}} = \frac{e^{-3.50-0.14x}}{1 + e^{-3.50-0.14x}} \]

µg ΣPCBs/g feed

pg TEQs/g feed

Lesion (%)

Lesion
Lesion 95% CI
EC20

Lesion
Lesion 95% CI
EC50

Lesion (%)

Lesion
Lesion 95% CI
EC20

Lesion
Lesion 95% CI
EC50